plasma membrane of the axon terminals contribute to the shaping of signaling to post-synaptic neurons.

3402-Pos Board B263

Acute Damage to Synaptic Ribbons Differentially Affects Evoked and Spontaneous Neurotransmitter Release from Rod Bipolar Cells

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Ribbon synapses are features of non-spiking tonic releasing cells of the retina and inner ear. These synapses are named for their electron dense synaptic ribbons, which tether an abundance of synaptic vesicles near release sites. Here, we used illumination of fluorescein-tagged ribbon-binding peptides to acutely damage synaptic ribbon function in mouse rod bipolar cells using fluorophore assisted light inactivation (FALI), while monitoring neurotransmitter release from a post-synaptic AII amacrine cell. Illumination of ribbon-targeted peptides, but not scrambled controls, revealed an immediate drop in the frequency and amplitude of mEPSCs at −60 mV without effect on the event kinetics, whereas the amplitude of the first EPSC evoked by a step to −10 mV for 100 ms was unaltered. These results suggest that two independent ribbon-associated pools of vesicles contribute to release at −60 mV and in response to steps to −10 mV. Our results are also consistent with a role for the ribbon in coordinating multivesicular release.

3403-Pos Board B264

A Catalytic Slot Model for Exocytosis with a Single Release Sensor

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Regulated secretion occurs on different timescales: fast (synchronous) release is mediated by high-release probability vesicles, whereas vesicles with low release probability contribute to slow (asynchronous) release. Current models of exocytosis incorporate parallel calcium-sensors, to account for the slow release remaining after deletion of the fast sensor. However, the identity of the slow calcium sensor remains elusive, and no molecular manipulation has been identified, which eliminates slow, but not fast release.

Using mathematical modeling we found that a simpler, sequential model with only a single release sensor suffices to describe previously obtained data in chromaffin cells and neurons: we suggest that during maturation vesicles associate with a catalyst at the release site. This catalyst facilitates priming by a calcium-dependent increase of the interconversion rate between un-primed and primed vesicles without changing the population of those states in equilibrium. We suggest that the calcium sensor for exocytosis (usually assumed to be synaptotagmin-1 or −2) regulates release by two distinct actions. It facilitates priming while clamping release in a calcium-independent manner. Calcium-binding to synaptotagmin relieves the clamp. In the absence of synaptotagmin spontaneous fusion depletes the primed vesicle state, thereby uncovering the upstream calcium-dependent catalysis step as the slow calcium sensor described in synaptotagmin nulls.

The model can explain salient observations, including calcium-dependence of pool sizes in chromaffin cells, fusion and recovery kinetics, and it accounts for a number of observations not easily explained in earlier models, including submaximal release (decreased fraction of fast/slow release at low calcium concentrations) and the phenotype of synaptotagmin knockouts when stimulated by high-frequency trains.

We suggest that slow release is not mediated by a parallel-acting, competing sensor, but by an upstream calcium-dependent catalysis step, making slow release a fundamental property of fast release itself.

3404-Pos Board B265

Observation of Two-Step Unzipping of a Single SNARE Complex by using Nano-Mechanical Measurement

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Soluble NSF Attachment Protein Receptors (SNARE) complex is known as the minimal machinery for synaptic vesicle exocytosis in neuronal communication. The conformational transition from ‘‘trans’’ to ‘‘cis’’ form of SNARE complex is an essential step of a Ca2+ triggered vesicle fusion to release neurotransmitters. Until recently, it has been debated whether the trans-SNARE complex is fully-zipped or partially-zipped before transition toward cis-form. We observe from nano-mechanical measurement by magnetic tweezer that a single soluble SNARE complex shows a sequential two-step unzipping, about 10nm each. It implies that trans-SNARE complex could be partially-zipped when force is applied like the hydration force between lipid bilayers. Other neuronal proteins might act on the unzipped domain to clamp trans-SNARE complex before conformational transition to cis-SNARE complex.

3405-Pos Board B266

Effects of Presynaptic Calcium Stores on Short-Term Synaptic Plasticity

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In reconstructions of hippocampal neuropil, Smooth Endoplasmic Reticulum (SER) appears in a majority of the presynaptic terminals. In the presence of ongoing electrical activity, Inositol Triphosphate Receptors (IP3Rs) on the SER initiate a positive feedback loop that can lead to release of calcium from the SER via an IP3-mediated pathway. We investigated how the presence of this additional source of calcium in addition to the Voltage Dependent Calcium Channels (VDCCs) can regulate synaptic transmission. We carried out 3D Monte Carlo simulations of the molecular interactions that govern transmitter release in a 1) Canonical CA3-CA1 synapse 2) Synapse reconstructed from serial section Transmission Electron Microscope images. The relatively simple geometry of CA3-CA1 synapses allows activity-dependent local calcium at the active zone and the related transmitter release profiles to be quantitatively analyzed. In paired-pulse stimulation, the presence of molecular pathways that regulate the calcium stores increased the calcium buffering capacity of the synapse, which decreased the initial release probability and enhanced paired-pulse facilitation. In contrast, a high-frequency stimulus could trigger the activation of presynaptic Metabotropic Glutamate Receptors (mGluRs) leading to IP3 production and ultimately to release of calcium from the SER. IP3Rs operated at a much slower time scale, on the order of seconds compared to the millisecond timescale of the VDCCs. This led to an increase in the basal level of intracellular calcium and enhanced transmitter release rates. We further explored the functional implications of the range of SER geometries observed in the synaptic ultrastructure and the effect of different arrangements between IP3Rs and VDCCs on synaptic plasticity. The synaptic ultrastructure precisely orchestrated the degree of facilitation and depression and the existence of presynaptic calcium stores provided the synapse with an additional intrinsic time scale that could be regulated by activity.

3406-Pos Board B267

Three Distinct Mechanisms of Neuro-Muscular Junction Excitation by Infrared Pulses

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Post-synaptic currents (PSCs) were recorded using whole cell voltage clamp to examine excitatory responses of the neuro-muscular junction to infrared pulses (1862nm, 200μs/pulse, 5-30 pulses/s, 20-1000 μJ/pulse over 125 nm^2). In wild type Caenorhabditis elegans, optical stimuli excited the post-synaptic cell by 1) immediate opto-mechanical triggering of pulse-by-pulse miniature excitatory currents (mLEC) with 0.7ms latency to peak and 2) relatively slow (τ~1.3s onset) thermodynamically driven reduction in a tonic outward rectified K^+ current (LTC). The same optical stimuli acted on the pre-synaptic neuron to 3) rapidly increase the rate of synaptic vesicle release and the rate of miniature PSCs (mPSCs). In addition, mPSC kinetics were increased with infrared stimulation resulting in a decrease in average charge per event from 52 to 32fC. The pulse-by-pulse mLECs were enhanced in muscle degenerin gain of function mutant (unc-105) suggesting the fast response was due to opto-mechanical activation of the degenerin stretch receptor. The slow tonic current (LTC) reversed at the K^+ equilibrium potential, exhibited a highly rectified outward conductance, and a thermal-dependent closure analogous to shaker related channels including K_v1.1. In the pre-synaptic neuron, the spontaneous rate of synaptic vesicle release and the laser-evoked increase was nearly eliminated in a loss of function mutation of the voltage insensitive cation leak channel (unc-77, nca-1). The increased mPSC rate (presynaptic action) and reduction in the tonic outward K^+ current (post-synaptic action) contributed in nearly equal proportions at −60mV holding potential and accounted for over 90% of the total laser-evoked PSC. [Supported by NIH R01 DC006685 & R01DC011481]

3407-Pos Board B268

Optical Activation or Silencing of Neural Activaty in Neurons that Regulate Energy Balance in the Hypothalamus

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